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(54) Title: MICROBIAL PRODUCTION OF CIS-DIHYDRODIOL AND PHENOL DERIVATIVES OF BENZOCYCLOBUTENE

(57) Abstract

A process for microbial conversion of benzocyclobutene to the corresponding 3,4-dihydrodiol followed by acid catalyzed dehydration to 3-hydroxybenzocyclobutene.

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TITLE

MICROBIAL PRODUCTION OF CIS-DIHYDRODIOL AND PHENOL DERIVATIVES OF BENZOCYCLOBUTENE

This invention was made with Government support under contract number F33615-89-C-5601 awarded by the United States Air Force. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

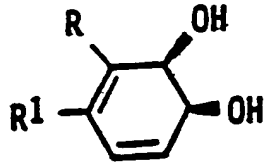
1.Field of the Invention:

The present invention relates to the bioconversion of benzocyclobutene (BCB) to the 3,4-cis-dihydrodiol compound and subsequent acid-catalyzed dehydration to form primarily 3-hydoxybenzocyclobutene. These novel compounds have utility as intermediates for the production of polymers.

BACKGROUND OF THE INVENTION

Formation of cis-dihydrodiols from various aromatic hydrocarbons by bacteria was first described by D. T. Gibson et al., Biochemistry, vol. 9, No. 7, 1973, p. 1626+, and p. 1631+ and vol. 12, No.8, 1973, p. 1520+. A cis-dihydrodiol intermediate has been found to be a common metabolite in the bacterial degradation of a variety of aromatic hydrocarbons, including benzene, toluene, naphthalene, biphenyl, ethylbenzene, benzoic acid, phthalic acid, anthracene and phenanthrene. U.S. Patent No. 4,508,822

discloses the preparation of dihydrodiols of the general formula :

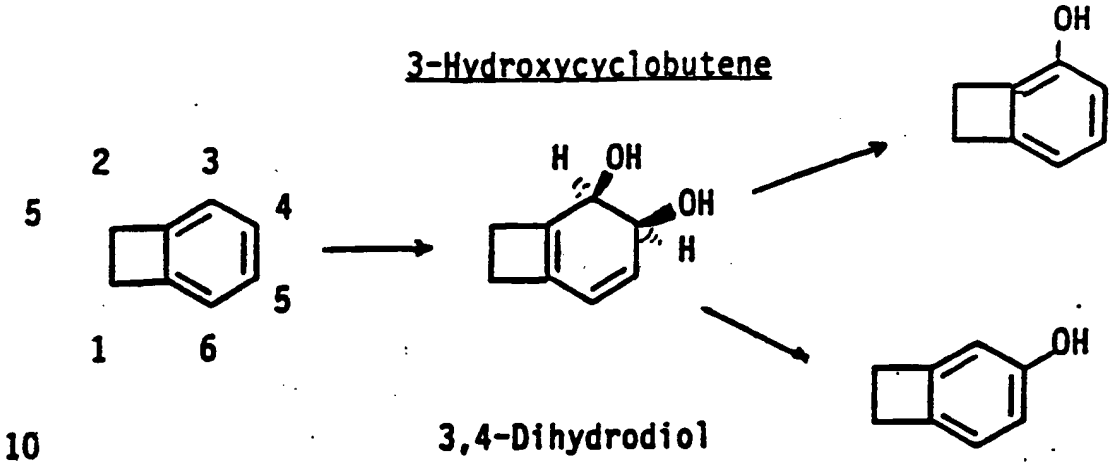


where R and R1 are substituents which may be the same or different, such as halogen, alkyl, etc. Generally such dihydrodiols are of the 2,3- dihydrodiol configuration. That is, the hydroxy groups are introduced directly adjacent to the ring substituent R. The only known exception to this general rule is the 4,5-dihydrodiol formed by some bacteria in the degradation of phthalic acid.

U.S. Patent 4,520,103 describes the formation of the 2,3-dihydrodiol of indole as an intermediate in the production of indigo.

SUMMARY OF THE INVENTION

The present invention relates to the formation of dihydrodiol resulting from bacterial bioconversion of the aromatic hydrocarbon benzocyclobutene. Mutant strains of Pseudomonas organisms capable of converting benzocyclobutene to the 3,4-dihydrodiol have been developed. The growth of mutant strain in the presence of benzocyclobutene results in the production of the 3,4-dihydrodiol intermediate of benzocyclobutene. Acid-catalyzed dehydration of the 3,4-hyhydrodial results in the formation of primarily 3-hydroxybenzocyclobutene and minor amounts of 4-hydroxybenzocyclobutene. The c rresponding sequential reactions are outlined below.



DETAILED DESCRIPTION OF THE INVENTION

Organisms capable of growth on a variety of aromatic hydrocarbons such as benzene, toluene, ethylbenzene and o-xylene were isolated from the environment by selective culture. Certain of the resulting isolates were found to partially metabolize benzocyclobutene to a mixture of dead-end metabolites, but were not able to grow on benzocyclobutene. Mutants lacking a functional diol dehydrogenase were obtained by mutagenesis with N-methyl-N-nitro-N-nitrosoguanidine, followed by ampicillin/cycloserine enrichment for mutants unable to grow on toluene. Diol dehydrogenase deficient mutants were identified by the accumulation of dihydrodiols upon exposure to various aromatic hydrocarbons.

Mutants created in several Pseudomonas strains, such as 18-36 American Type Culture Collection (ATCC 55196), 18-803 (ATCC55197), 34-35 (ATCC55198), 35-50 (ATCC55199) and 44-12 (ATCC55200), convert benzocyclobutene to the corresponding 3,4-dihydrodiol compound. Th dihydrodiol at a concentration of two hundred to four thousand parts per million in aqueous solution is dehydrated by

addition of a mineral acid such as hydrochloric acid or sulfuric acid to a concentration of 0.1 N to 8 N, preferably 1.0 to 5N at a temperature of 20° to 50°C for 15 minutes to 20 hours, preferably 1 to 10 hours. The resulting phenols can be recovered such as by extraction with water immiscible, polar organic solvents, such as ethyl acetate, methyl ethyl ketone, etc. The resulting phenols are generally in the range of 85-90 wt.% 3-hydroxybenzocyclobutene, with the remainder as 4-hydroxybenzocyclobutene.

Example 1

Pseudomonas strain 18-803 (ATCC55197) is grown in 125 ml baffled Erlenmeyer flasks on a minimal salts medium containing 1.0 wt % succinate, with benzocyclobutene supplied to the culture as a vapor, after 24 hours incubation on a rotary shaker, operated at 150 rpm and 30°C, the culture is centrifuged and the resulting broth acidified with HCl to a final concentration of 1.0 N. After 2 hours the acidified broth is extracted with an equal volume of ethyl acetate. The phenols present in the organic phase are assayed by gas chromatography.
3-Hydroxybenzocyclobutene is present at 670 ppm, and 4-hydroxybenzocylobutene at 84 ppm.

Example 2

Pseudomonas strain 18-36 (ATCC55196) is grown in 125 ml baffled Erlenmeyer flasks on a minimal salts medium containing 1.0 wt % succinate, with benzocyclobutene supplied to the culture as a vapor. After 24 hours incubation on a rotary shaker, operated at 150 rpm and 30°C the culture is centrifuged and the resulting broth acidified with HCl to a final concentration of 1.0 N. After 24 hours at 30°C the

acidified broth is extracted with an equal volume of ethyl acetate. The phenols present in the organic phase are assayed by gas chromatography. 3-Hydroxybenzocyclobutene is present at 336 ppm, and 4-hydroxybenzocyclobutene at 61 ppm.

Example 3

Pseudomonas strain 34-35 (ATCC55198) is grown in 125 ml baffled Erlenmeyer flasks on a minimal salts medium containing 1.0 wt % succinate, with benzocyclobutene supplied to the culture as a vapor. After 24 hours incubation on a rotary shaker, operated at 150 rpm and 30°C the culture is centrifuged and the resulting broth acidified with HCl to a final concentration of 1.0 N. After 24 hours at 30°C the acidified broth is extracted with an equal volume of ethyl acetate. The phenols present in the organic phase are assayed by gas chromatography. 3-Hydroxybenzocyclobutene is present at 221 ppm, and 4-hydroxybenzocyclobutene at 36 ppm.

Example 4

Pseudomonas strain 35-50 (ATCC55199) is grown in 125 ml baffled Erlenmeyer flasks on a minimal salts medium containing 1.0 wt % succinate, with benzocyclobutene supplied to the culture as a vapor. After 24 hours incubation on a rotary shaker, operated at 150 rpm and 30°C the culture is centrifuged and the resulting broth acidified to a final concentration of 1.0 N. After 24 hours at 30°C the acidified broth is extracted with an equal volume of ethyl acetate. The phenols present in the organic phase are assayed by gas chromatography. 3-Hydroxybenzocyclobutene is present at 297 ppm. 4-Hydroxybenzocyclobutene is present at 40 ppm.

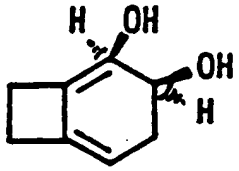
Example 5

Pseudomonas strain 44-12 (ATCC55200) is grown in 125 ml baffled Erlenmeyer flasks on a minimal salts medium containing 1.0 wt % succinate, with benzocyclobutene supplied to the culture as a vapor. After 24 hours incubation on a rotary shaker, operated at 150 rpm and 30°C, the culture is centrifuged and the resulting broth acidified to a final concentration of 1.0 N. After 24 hours at 30°C the acidified broth is extracted with an equal volume of ethyl acetate. The phenols present in the organic phase are assayed by gas chromatography. 3-Hydroxybenzocyclobutene is present at 278 ppm. 4-Hydroxybenzocyclobutene is present at 39 ppm.

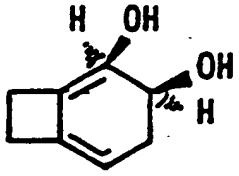
CLAIMS

1. 3-Hydroxybenzocyclobutene.

2. A dihydrodiol of the formula



3. A process for production of a dihydrodiol compound of the formula



comprising growing a mutant strain of Pseudomonas in a growth medium at 25° to 35°C and at a pH in the range of 6 to 8, in the presence of oxygen or an oxygen containing gas wherein benzocyclobutene is supplied to the growing mutant strain.

4. The process of claim 3 wherein the mutant strain of Pseudomonas is ATCC55196, ATCC55197, ATCC55198, ATCC55199, or ATCC55200.

5. The process of claim 4 wherein the dihydrodiol compound is treated at 20° to 50°C with an aqueous acid solution containing 0.1 to 8 N mineral acid for 15 minutes to 20 hours to form 3-hydroxybenzocyclobutene.

6. The process of claim 5 wherein the 3-hydroxybenzocyclobutene is extracted from the acidified solution with a water immiscible, polar organic solvent.

7. The process of claim 6 wherein the product is principally 3-hydroxybenzocyclobutene.

8. The process of claim 7 wherein the strain is Pseudomonas ATCC55196.

9. The process of claim 7 wherein the strain is Pseudomonas ATCC55197.

10. The process of claim 7 wherein the strain is Pseudomonas ATCC55198.

11. The process of claim 7 wherein the strain is Pseudomonas ATCC55199.

12. The process of claim 7 wherein the strain is Pseudomonas ATCC55200.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/09213

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12P 7/22, C07C 39/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12P, C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chemical Society Journal. Perkin transactions I, Volume 8, 1980, Omar Abou-Teim et al., "Benzocyclobutenes. part 5.1 Synthesis of 4-Hydroxy-, 4,5-Dihydroxy-, and 3, 6-Dihydroxy-benzocyclobutene-1,2-dione (Benzologues of Semisquaric and Squaric Acid)", page 1841 - page 1846, see example 7, p. 1841	1-12
A	J. Org. Chem., Volume 47, 1982, Michael S. South et al., "Practical Multigram Syntheses of Benzocyclobutenediones", page 3816 - page 3821, see p 3816	1-12

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1993	11.03.93
Name and mailing address of the ISA/	Authorized officer

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/09213

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
A	US, A, 4508822 (S. C. TAYLOR), 2 April 1985 (02.04.85)	3-12